

## POLYCLONAL ACTIVATION OF HUMAN B LYMPHOCYTES BY Fc FRAGMENTS

### I. Characterization of the Cellular Requirements for Fc Fragment-mediated Polyclonal Antibody Secretion by Human Peripheral Blood B Lymphocytes\*

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The use of nonspecific polyclonal activation of lymphocytes has long been used as a model for the study of antigen-induced responses. Essential to the study of bone marrow-derived (B) cell activation is the ability to reproducibly induce and detect antibody production *in vitro*. Polyclonal activators offer the unique ability to stimulate a greater percentage of the total B cell population. In addition to generating a greater understanding of B cell physiology *in vitro*, tests that can be used to selectively analyze human B and thymus-derived (T) cell function represent important tools for evaluating the immunological status of patients with various diseases.

Pokeweed mitogen (PWM)<sup>1</sup> has been extensively used to trigger the *in vitro* production and secretion of immunoglobulin (Ig) by human B cells. Workers have demonstrated Ig production by measuring intracytoplasmic Ig levels (1), the presence of Ig in culture supernates (2), and by local hemolysis in gel (3, 4). In addition to plant lectins, substances derived from bacteria (5, 6) and viruses (7) have been shown to be potent polyclonal activators for human B cells. It has recently been shown that the Fc region of Ig, whether in the form of an Fc fragment (8–10), aggregated Ig (AHGG) (11), or an immune complex (12) can induce murine splenic B cells to proliferate and differentiate to polyclonal antibody-secreting cells. Fc fragment-induced polyclonal antibody production requires the presence of both macrophages and T cells (10).

The ability of Fc fragments to stimulate murine B cells prompted investigation for their ability to serve as a probe for the study of human B cell activation. The studies in this report demonstrate that Fc fragments can serve as a potent polyclonal human B cell activator. Furthermore, the cellular requirements for such B cell activation are documented.

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<sup>1</sup> Abbreviations used in this paper: AHGG, aggregated human gamma globulin; FCS, fetal calf serum; PBS, phosphate-buffered saline; PBL, peripheral blood lymphocytes; PFC, plaque-forming cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SRBC, sheep erythrocytes.

### Materials and Methods

*Source of Peripheral Blood Lymphocytes (PBL).* Venous blood was collected in heparinized Vacutainer tubes (Becton, Dickinson & Co., Oxnard, Calif.) from healthy adult donors of both sexes, ranging in age from 22–53 mo.

*Source of Spleen Cells.* Normal spleen was obtained from a trauma patient through the courtesy of the Department of Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

*Preparation of PBL.* Heparinized peripheral blood was diluted with 2 vol of phosphate-buffered saline (PBS), 0.001 M phosphate, pH 7.2, and 0.15 M NaCl. The diluted blood was then centrifuged at 2,500 rpm for 10 min to deplete platelets (13). The cell pellet was resuspended to the original volume and the PBL were isolated by Ficoll-Paque density gradient centrifugation. Briefly, 30 ml of diluted blood was layered into 15-ml Ficoll-Paque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). The gradient was then centrifuged at 2,200 rpm for 30 min at room temperature. The PBL at the interface were collected and washed once in PBS + 10% fetal calf serum (FCS) (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) before use. This procedure yielded between  $1-2 \times 10^6$  mononuclear cells/ml whole blood.

*Isolation of Peripheral Blood B and T Cells.* PBL suspensions were enriched for B and T cells by the neuraminidase-treated sheep erythrocyte (SRBC) rosetting technique (14). The nonrosetting cells were defined as B cell enriched and the rosetting cells as T cell enriched. The T cell populations were subjected to 2,000 rad of irradiation (Gamma Cell 40; Atomic Energy of Canada Ltd., Ottawa, Canada) before use in the in vitro antibody response cultures.

*Preparation of Fc Fragments.* A human IgG<sub>1</sub> myeloma protein was a gift from Dr. Hans L. Spiegelberg, Scripps Clinic and Research Foundation. The IgG<sub>1</sub> was purified by ammonium sulfate fractionation followed by DEAE-cellulose chromatography with 0.01 M phosphate buffer, pH 8, used as the eluent. Fc fragments were obtained by digestion of IgG<sub>1</sub> with papain (Sigma Chemical Co., St. Louis, Mo.) in the presence of L-cysteine (Sigma Chemical Co.) and EDTA (J. T. Baker Chemical Co., Phillipsburg, N. J.) for 5 h (15). Following digestion, the material was chromatographed on Sephadex G-100 (Pharmacia Fine Chemicals) to remove any undigested IgG. The Fc and Fab fragments were then separated from each other by DEAE chromatography (16).

#### *Preparation of Fc Subfragments*

(a) PREPARATION OF ADHERENT CELL SUPERNATE. PBL were given 2,000 rad irradiation and resuspended to a concentration of  $5 \times 10^6$  cells/ml RPMI 1640 (Flow Laboratories Inc., Rockville, Md.) supplemented with 2 mM L-glutamine, 1% BME vitamins (Gibco Laboratories, Grand Island Biological Co.), 100 U penicillin and 100 µg streptomycin (Microbiological Associates, Bethesda, Md.), and 10% FCS. 1-ml samples were allowed to adhere to tissue culture dishes (3001; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for 1 h in an atmosphere of 5% CO<sub>2</sub> at 37°C. The cells were washed extensively to remove nonadherent cells before the addition of Fc fragments. 100 µg of Fc in 1 ml of RPMI 1640, supplemented as described above except without serum, was added to the adherent cell monolayer. Supernatant material was collected after 1 h and centrifuged at 2,000 rpm before chromatographic separation.

(b) CHROMATOGRAPHIC SEPARATION. The adherent cell supernate was chromatographed on Sephadex G-75 (Pharmacia Fine Chemicals) column (1.2 × 60 cm) with a bed volume of 40 ml. The material was eluted with saline at a flow rate of 20 ml/h, and 1.0-ml samples were collected. The column was calibrated with the following standards: blue dextran (>75,000 mol wt) (Pharmacia Fine Chemicals), deoxyribonuclease (31,000 mol wt) (Sigma Chemical Co.), and lysozyme (14,300 mol wt) (Sigma Chemical Co.). The molecular weight of the biologically active material was calculated from a plot of the elution volumes vs. the logarithm of the molecular weight of the standards.

(c) AFFINITY CHROMATOGRAPHY. Pooled column fractions containing the polyclonally active material were passed over an anti-Fc affinity column, as described previously (10, 17).

*Depletion of Macrophages*

(a) DEPLETION BY SEPHADEX G-10 FILTRATION. PBL enriched for B cells were filtered through columns of Sephadex G-10 (Pharmacia Fine Chemicals) by the method of Ly and Mishell (18). Briefly,  $2 \times 10^7$  cells in 2 ml RPMI 1640 supplemented with 5% FCS were filtered through a column containing 9 ml of Sephadex G-10 that was previously equilibrated with RPMI 1640 + 5% FCS.

(b) DEPLETION BY PLASTIC ADHERENCE. B cell-enriched populations were adjusted to  $2 \times 10^7$  cells/ml, and 1 ml was allowed to adhere to plastic (3001; Falcon Labware, Div. of Becton, Dickinson & Co.) for 1 h, at which time the nonadherent cells were carefully removed.

*PBL Proliferation.* Unseparated PBL, B cell-enriched, and T cell-enriched populations were resuspended to  $5 \times 10^6$  cells/ml RPMI 1640 (Flow Laboratories Inc., Rockville, Md.) supplemented with 2 mM L-glutamine, 1% BME vitamins (Gibco Laboratories, Grand Island Biological Co.), 100 U penicillin and 100 µg streptomycin (Microbiological Associates), and 10% FCS (Gibco Laboratories, Grand Island Biological Co.).  $5 \times 10^5$  cells were cultured with PWM (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.), phytohemagglutinin (PHA; Difco Laboratories, Detroit, Mich.), or Fc fragments in 0.2-ml final volume in flat-bottomed microtiter plates (3042 Microtest II; Falcon Labware) for 3 d at 37°C in an atmosphere of 5% CO<sub>2</sub> in air. The amount of proliferation was assessed by incorporation of 1 µCi of [<sup>3</sup>H]thymidine (New England Nuclear, Boston, Mass.) during the last 24 h of culture.

*Polyclonal Antibody Production by PBL.* B cell- and T cell-enriched populations were resuspended as described above and mixed together at various ratios in a final volume of 0.3 ml in round bottomed Cooke microtiter plates (Dynatech Laboratories Inc., Alexandria, Va.). The plates were incubated from 2-8 d at 37°C in an atmosphere of 5% CO<sub>2</sub> in air. Triplicate cultures were harvested and assayed for a plaque-forming response, as described below.

*Plaque-forming Responses.* The in vitro cultures were assayed for direct or IgM plaque-forming cells (PFC) to SRBC by coupling SRBC onto poly-L-lysine-coated flat bottomed microtiter plates (19) or to total Ig-secreting cells by a modification of the protein A plaque assay (20). Amplifying sera used in the protein A assay recognized human IgG + IgM + IgA and was used at a dilution of 1:100.

## Results

*Fc Fragment-induced Proliferation.* Recent studies have established that Fc fragments derived from mammalian Ig induce murine splenic B cells to proliferate (8, 9, 17). To determine whether Fc also had the capacity to stimulate human PBL to proliferate, intact PBL, B cell-enriched (SRBC rosette negative), and T cell-enriched (SRBC rosette positive) populations were assessed. The results in Table I indicate that untreated PBL are induced to proliferate in response to Fc fragments (ninemfold stimulation) compared with background controls, whereas Fab fragments are unable to induce a proliferative response. Moreover, Fab fragments did not induce any proliferation in PBL cultures over a wide range of concentrations (data not shown). Untreated PBL also responded to the T cell mitogen PHA (51-fold stimulation) and to PWM (25-fold stimulation), which stimulate both B and T cells (Table I). When the PBL population was enriched for B cells, the response to Fc fragments was enhanced (9- vs. 15-fold), the PHA response was reduced (51- vs. 7-fold), and the PWM response was slightly reduced (25- vs. 20-fold) compared with intact PBL cultures. In contrast, when the PBL population was enriched for T cells, the Fc fragment response was reduced to near background levels (9- vs. 2-fold). The PHA response was enhanced (51- vs. 112-fold), and the PWM response was approximately the same (25- vs. 24-fold) compared with untreated control responses. In neither the B cell- nor T cell-enriched populations did Fab fragments induce a proliferative response. These results indicate that, as in the murine model, Fc fragments stimulate human B cells to proliferate.

TABLE I  
*Fc Fragment-mediated Activation of Human PBL*

| PBL treatment                        | Stimulator | $[^3\text{H}]$ Thymidine uptake<br>± SE* |
|--------------------------------------|------------|--|
| <i>cpm × 10<sup>-3</sup>/culture</i> |            |  |
| None                                 | —          | 2,001 ± 110                              |
| None                                 | Fab‡       | 2,210 ± 1,099                            |
| None                                 | Fc§        | 19,250 ± 2,111                           |
| None                                 | PWM        | 49,986 ± 3,171                           |
| None                                 | PHA¶       | 102,666 ± 5,280                          |
| B cell-enriched**                    | —          | 1,705 ± 91                               |
|                                      | Fab        | 1,910 ± 600                              |
|                                      | Fc         | 26,814 ± 3,157                           |
|                                      | PWM        | 35,000 ± 1,660                           |
|                                      | PHA        | 11,519 ± 1,200                           |
| T cell-enriched††                    | —          | 1,213 ± 136                              |
|                                      | Fab        | 1,717 ± 296                              |
|                                      | Fc         | 2,000 ± 200                              |
|                                      | PWM        | 34,200 ± 6,700                           |
|                                      | PHA        | 145,922 ± 5,339                          |

\* The response was measured on day 3 of culture.

‡ 1.0 µg Fab/culture.

§ 1.0 µg Fc/culture.

|| 10 µg PWM/culture.

¶ 2 µg PHA/culture.

\*\* Sheep erythrocyte rosette-negative cells (B cells).

†† Sheep erythrocyte rosette-positive cells (T cells).

*Fc Fragment-induced Polyclonal Antibody Response.* Because Fc fragments induce human peripheral blood B cells to proliferate, experimentation was conducted to determine whether Fc could stimulate B cells to secrete polyclonal antibody. It was observed that B cells could be stimulated to secrete polyclonal antibody in the presence of Fc fragments (Fig. 1). 1 µg Fc/culture was found to induce the maximum response as measured by total Ig secretion (protein A plaque assay) and by direct anti-SRBC response. To determine the optimal day for assaying the in vitro polyclonal response, cultures were plaqued from day 2 to day 7 of culture. Day 6–7 was found to be the optimum time for assaying total Ig secretion and day 5 was optimum for the anti-SRBC plaque-forming response (Fig. 2).

Preliminary experiments indicated that intact PBL populations were inconsistent in their ability to produce the polyclonal antibody response (E. L. Morgan, unpublished results). It has been reported in the PWM system that irradiation of T cells before their addition to B cells resulted in enhanced antibody responses (10). Therefore, varying numbers of irradiated T cells were added to the B cell-enriched (B cells + macrophages) populations and the polyclonal antibody response was measured. The results show that the B cell-enriched population was unable to secrete polyclonal antibody in response to Fc in the absence of T cells. The addition of twice the number of T cells to B cells (2:1) resulted in the maximum polyclonal antibody response when assayed by the protein A assay, and an equal number of T cells to B cells produced the optimal anti-SRBC response (Fig. 3). It is interesting to note that a 3:1 T cell to

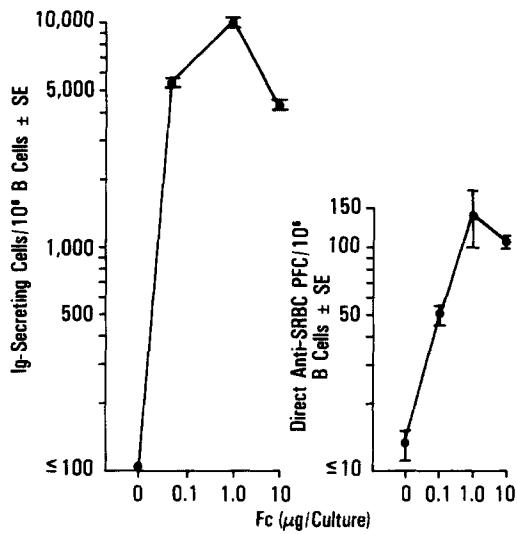


FIG. 1. Dose response of the Fc fragment-mediated polyclonal antibody response by human PBL. Total Ig secretion was measured on day 6 from cultures that contained  $1 \times 10^6$  B cells and  $2 \times 10^6$  T cells. The anti-SRBC response was measured on day 5 from cultures that contained  $1 \times 10^6$  B cells and  $1 \times 10^6$  T cells.

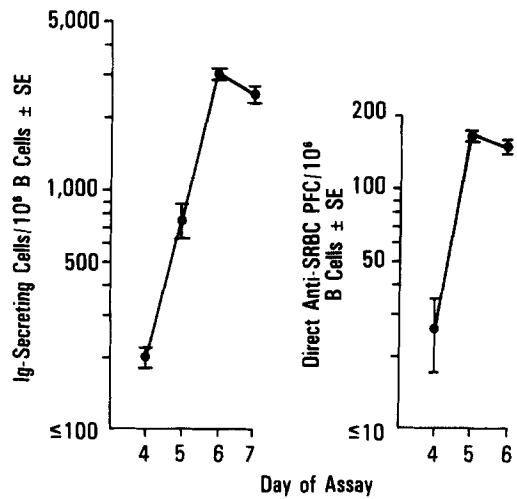


FIG. 2. Kinetics of the Fc fragment-mediated polyclonal antibody response by human PBL.  $1 \mu\text{g}$  Fc/culture was used, and the response was measured from day 4 to day 7. The B:T cell ratios were identical to those in Fig. 1.

B cell ratio completely abrogated the anti-SRBC response, whereas total Ig secretion was only marginally reduced.

To examine the variability of PBL from different individuals to be stimulated by Fc fragments, experiments were performed comparing 15 individuals for their ability to respond to Fc fragments. The results in Fig. 4 reveal that, as observed with PWM responses (20), marked variation exists from individual to individual. The mean response for total Ig secretion was 4,500 PFC and for the direct anti-SRBC response was 145 PFC.

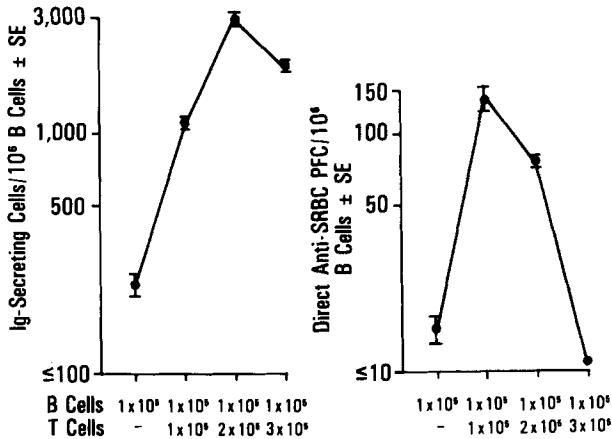


FIG. 3. Optimal B:T cell ratio in the Fc fragment-mediated polyclonal antibody response by human PBL. 1  $\mu$ g Fc/culture was used, the protein A response was measured on day 6, and the anti-SRBC response was measured on day 5.

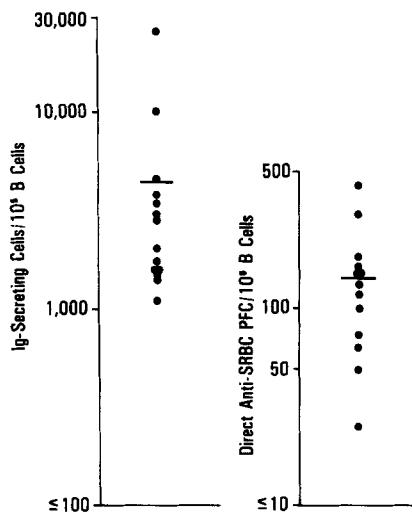


FIG. 4. Comparison of the Fc fragment-mediated polyclonal antibody response from different individuals. 1  $\mu$ g Fc/culture was used, and the B:T cell ratios were identical to those described in Fig. 1.

Because the murine model used splenic lymphocytes, it became important to determine whether lymphocytes derived from human spleen were capable of mounting a polyclonal antibody response to Fc fragments. Normal spleen from a trauma patient was found to produce a significant polyclonal anti-SRBC PFC response in the presence of Fc fragments (Table II). These results indicate that splenic B cells as well as peripheral blood B cells can be stimulated by Fc fragments. However, the optimal conditions for generation of the splenic polyclonal antibody response are currently unknown because of the limited availability of normal spleen, and therefore must await further investigation.

*Fc Subfragment-induced Polyclonal Antibody Response.* Macrophages have been shown to be essential for the Fc fragment-mediated proliferative (9) and polyclonal antibody

TABLE II  
*Fc Fragment-mediated Polyclonal Antibody Response of Human Splenic Lymphocytes*

| Spleen cells*         | Fc | Direct anti-SRBC PFC/ $10^6$<br>± SE‡ |
|-----------------------|----|---------------------------------------|
| $\mu\text{g/culture}$ |    |                                       |
| +                     | -  | 15 ± 7                                |
| +                     | 10 | 390 ± 14                              |

\*  $5 \times 10^5$  cells/0.3 ml culture volume.

‡ The response was measured on day 3 of culture.

TABLE III  
*Inability of Macrophage-depleted PBL to Respond to Fc Fragments*

| Treatment |          | Fc§ | Ig-secreting cells/ $10^6$ B cells ± SE |
|-----------|----------|-----|---|
| B cells*  | T cells‡ |     |   |
| None      | None     | -   | 305 ± 48                                |
| None      | None     | +   | 1,322 ± 110                             |
| G-10      | None     | -   | 50 ± 13                                 |
| G-10      | None     | +   | 90 ± 47                                 |
| Adherence | None     | -   | 85 ± 26                                 |
| Adherence | None     | +   | 115 ± 11                                |

\*  $1 \times 10^5$  B cells/culture.

‡  $1 \times 10^5$  T cells/culture.

§ 1  $\mu\text{g}$  Fc/culture.

|| The response was measured on day 6 of culture.

(10) responses in the murine model. To assess the role of peripheral blood monocytes in the Fc response, PBL populations were depleted of monocytes by Sephadex G-10 filtration or by plastic adherence before culturing with Fc fragments. The results show that either depletion method is equally efficient in that the polyclonal antibody response was reduced to background levels (Table III). These results indicate that adherent monocytes are required for the Fc-mediated polyclonal antibody response.

To further define the role of the adherent monocytes in the Fc-mediated polyclonal antibody response, irradiated adherent cells were cultured with or without Fc fragments for 1 h; the supernatant material was then collected and assayed on monocyte-depleted cultures. The results indicate that the Fc supernate could stimulate monocyte-depleted cultures, whereas Fc fragments were unable to stimulate a polyclonal response (Fig. 5). Moreover, the polyclonal antibody response obtained with Fc supernate was equivalent to that obtained with Fc fragments in cultures containing monocytes. Supernatant material collected from monocytes cultured in the absence of Fc fragments was nonstimulatory (Fig. 5). Because murine macrophages digest Fc fragments into biologically active Fc subfragments (17), the human monocyte Fc supernate was chromatographed on Sephadex G-75 and the column fractions were assessed for their ability to stimulate polyclonal antibody production in monocyte-depleted PBL cultures. The results in Fig. 6 reveal that the biologically active component in the Fc supernate has ~19,000 mol wt. No biological activity was found to be associated with the original Fc material because the cell population was depleted of monocytes before culturing. To further characterize the 19,000 mol wt active

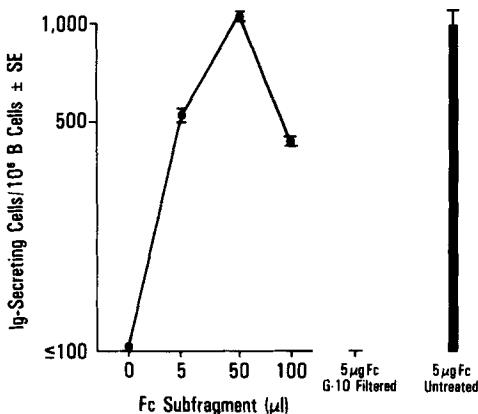


FIG. 5. Ability of human adherent monocytes to digest Fc fragments into polyclonally active Fc subfragments. Increasing amounts of Fc subfragment were added to monocyte-depleted (G-10 filtered) cell populations, and the response was measured on day 6 of culture. The T:B cell ratio was the same as in Fig. 1. Normal supernate <100 PFC.

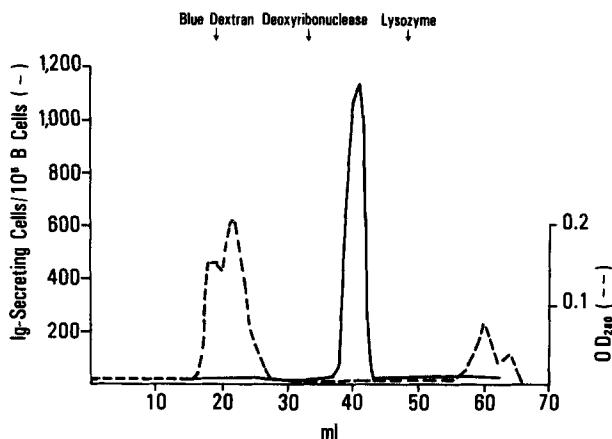


FIG. 6. Sephadex G-75 chromatographic separation of Fc-adherent cell supernate. Responder cell populations were Sephadex G-10 filtered before culture, and the response was measured on day 6 of culture.

material, it was filtered through an anti-Fc fragment affinity column and the effluent and eluate were assessed for polyclonal activity. The results in Table IV show that the 19,000 mol wt component induces a pronounced polyclonal response compared with the control culture (2,096 vs. 56 PFC). When this material was subjected to affinity-column filtration, the polyclonal activating material was found in the eluate, indicating that it bound to, and could be eluted from, the anti-Fc affinity column (1,912 PFC). One passage through the column reduced the polyclonal response from 2,096 to 272 PFC (87% reduction). These results indicate that the 19,000 mol wt polyclonal activation in the Fc monocyte supernate is derived from the Fc fragment.

### Discussion

Human B cells derived from peripheral blood are induced to proliferate and secrete polyclonal antibody upon stimulation with Fc fragments derived from human Ig. The

TABLE IV  
*Recovery of the Polyclonal-activating Material from an Anti-Fc Affinity Column*

| Supernate source | Treatment               | Ig-secreting cells/<br>$10^6$ * B cells $\pm$ SE |
|------------------|-------------------------|--|
| None             | None                    | 56 $\pm$ 6                                       |
| Fc-monocyte‡     | None                    | 2,096 $\pm$ 48                                   |
| Fc-monocyte      | Anti-Fc column effluent | 272 $\pm$ 12                                     |
| Fc-monocyte      | Anti-Fc column eluate   | 1,912 $\pm$ 89                                   |
| Monocyte§        | None                    | 60 $\pm$ 9                                       |

\* The response was measured on day 6 of culture.

‡ The 19,000 mol wt fraction from the Sephadex G-75 chromatographic separation of Fc monocyte supernate.

§ Supernate from monocytes incubated without Fc fragments.

addition of Fc fragments, but not Fab fragments or intact Ig, to in vitro lymphocyte cultures results in a significant polyclonal antibody response measured by either anti-SRBC or total Ig plaque-forming responses.

The Fc fragment-mediated human polyclonal antibody, but not the proliferative response, requires the presence of T cells. The optimum B cell to T cell ratio was found to be 1:2 for the protein A response and 1:1 for the anti-SRBC response. Preliminary experiments revealed that antibody responses were significantly higher when irradiated (2,000 rad) T cells were used. This finding is consistent with that of Fauci et al. (20) and Dosch et al. (21), who have shown that human T helper cell activity is more resistant to irradiation than T suppressor cell activity. The requirement for T cells in the human Fc polyclonal antibody response is reminiscent of the results observed in the murine model (10, 12, 22, 23). In the murine system, T cells provide a second or differentiative signal to the B cell (10), which, in conjunction with the Fc proliferative signal, induces the polyclonal antibody response. Moreover, it was shown by Thoman et al. (22, 23) that the T cell requirement can be substituted for by a soluble T cell factor (Fc)TRF derived from T cells after stimulation with Fc fragments. Similar T cell requirements have been reported for polyclonal antibody production by human B cells to PWM (24-27). The pathway for polyclonal B cell activation by PWM may be different from that of Fc fragment because PWM stimulates both B and T cells to proliferate, whereas Fc fragments induce only B cells to proliferate. An additional example of T cell participation in B cell polyclonal activation was reported by Shinohara and Kern (28), who found that when T cells were added to rabbit B cells, an enhanced lipopolysaccharide-induced polyclonal antibody response occurred. Goodman and Weigle (29) have extended this observation to the murine model, showing that T cells produce a modest enhancement of the lipopolysaccharide polyclonal antibody response. More recently, Levitt et al. (30) found that under appropriate culture conditions, the addition of T cells to lipopolysaccharide-stimulated human peripheral blood B cells resulted in the development of IgG and IgA plasma cells and an increase in IgM plasma cells.

Adherent monocytes are required in addition to T cells to obtain an Fc fragment-mediated polyclonal antibody response by human B cells. The accessory cell population was characterized as being plastic adherent, resistant to irradiation, SRBC-rosette negative, and depleted by Sephadex G-10 filtration. The function of the monocytes

is to cleave the Fc fragments into biologically active Fc subfragments. Incubation of Fc fragments with adherent monocytes results in a supernate that has the capacity to induce a polyclonal antibody response in monocyte-depleted cultures. Sephadex G-75 chromatographic separation of the supernate revealed that the biologically active material had ~19,000 mol wt. That the 19,000 mol wt subfragment was derived from the original Fc fragment was proven by the ability of the subfragment to be bound to and eluted from an anti-Fc affinity column. The requirement for T cells was not bypassed by the use of Fc subfragments. Murine splenic macrophages have been shown to be essential for the Fc fragment-mediated proliferative (9, 17) and polyclonal (10) responses. Murine macrophage digestion of Fc results in the production of Fc subfragments with ~14,000 mol wt (17). The significance in the size differences between Fc subfragments generated with human monocytes and murine macrophages is unknown and is currently being studied. Passwell et al. (31) have recently reported that Fc fragments increase endogenous production of prostaglandin E by human monocyte monolayers. In addition, they observed (31) that Fc induces the secretion of IgM into culture supernates, and that the IgM secretion was independent of monocytes. The apparent difference between the results reported here and those of Passwell et al. (31) could be explained by the culture conditions used. The requirements for monocytes in the PWM-induced polyclonal antibody response have also been a point of contention. It has been reported that the PWM polyclonal response is both independent of (32, 33) and dependent on (34, 35) the presence of monocytes. Rosenberg and Lipsky (36) found that the monocyte requirement of the PWM response was dependent upon the cell density in culture. The greatest degree of monocyte dependence occurred when limited cell-to-cell interaction occurred (i.e., flat-bottomed wells or smaller numbers of cells per culture).

The observation that Fc fragments (8–10), aggregated human gamma globulin (11), and antigen-antibody complexes (12) all trigger B cells to secrete polyclonal antibody, and the fact that in all cases the biologically active mediator is a small subfragment derived from the Fc molecule, strongly suggests that the pathways of activation are the same. Regulation of both *in vivo* and *in vitro* lymphocyte activation via immune complexes could occur by the generation of biologically active Fc subfragments. That Fc fragments have the capacity to regulate both *in vivo* and *in vitro* humoral (37) and T cell-mediated (38) responses has recently been reported. Fc fragments were shown to potentiate antigen-specific antibody formation and to enhance antigen and allogeneic cell-induced T cell proliferation. The fact that Fc fragments can function as a polyclonal T cell activator for human B cells does not necessarily mean that Fc can act as a human adjuvant. Experimentation is currently in progress to answer this question.

### Summary

Fc fragments derived from human immunoglobulin were found to be capable of inducing both a proliferative and polyclonal antibody response in human peripheral blood lymphocyte cultures. The cell population proliferating in response to Fc fragments belongs to the B cell lineage. Expression of polyclonal antibody formation requires the presence of both adherent monocytes and T cells. The role of the monocyte is to enzymatically cleave the Fc fragment into 19,000 mol wt Fc subfragments that are then able to induce polyclonal antibody secretion. Stimulation of

polyclonal antibody production by Fc subfragments occurs in the absence of adherent monocytes but still requires the presence of T cells.

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